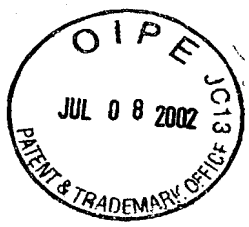


June 27, 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of D. Goring, N. Silva, Y. Haffani
U.S. Application No. 10/086,464
Filed February 28, 2002
Proline-rich Extensin-like Receptor Kinases

Group No. 1645
Examiner NYA

(Atty. Docket No. P 25,762-A USA)

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231, on Thursday, June 27, 2002.

Gene J. Yao

Commissioner for Patents
Washington, D.C. 20231

THIRD PRELIMINARY AMENDMENT UNDER 37 CFR § 1.115

Sir:

Applicants request entry of the following amendments.

In the Description

Please amend the paragraph, commencing at page 16, line 12, as follows.

Figure 11. Shows the nucleic acid molecules of [SEQ ID NOS:6 & 7] and the amino acid sequence of [SEQ ID NO:8].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number AAC98010

A) Genomic Sequence [SEQ ID NO:6]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS:7 & 8]. The transmembrane domain is underlined.

Please amend the paragraph, commencing at page 16, line 19, as follows.

Figure 12. Shows the nucleic acid molecules of [SEQ ID NOS:9 & 10] and the amino acid sequence of [SEQ ID NO:11].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number AAD15491

A) Genomic Sequence [SEQ ID NO:9]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS:10 & 11]. The transmembrane domain is underlined.

Please amend the paragraph, commencing at page 16, line 26, as follows.

Figure 13. Shows the nucleic acid molecules of [SEQ ID NOS:12 & 13] and the amino acid sequence of [SEQ ID NO:14].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number CAA18823.

A) Genomic Sequence [SEQ ID NO:12]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS:13 & 14]. The transmembrane domain is underlined.

Please amend the paragraph, commencing at page 17, line 4, as follows.

Figure 14. Shows the nucleic acid molecules of [SEQ ID NOS:15 & 16] and the amino acid sequence of [SEQ ID NO:17].
In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number CAA18590
A) Genomic Sequence [SEQ ID NO:15]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.
B) Translation of the predicted open reading frame [SEQ ID NOS: 15 & 16]. The transmembrane domain is underlined.

Please amend the paragraph, commencing at page 52 line 11, as follows.

The isolation of novel *Brassica napus* receptor kinases relied upon the newly constructed cDNA library and involved *in vivo* mass excision of the pBluecsript phagemids from the Uni-ZAP XR vectors as outlined by the manufacturer (Stratagene, La Jolla, CA). Following efficient mass excision, phagemid DNA was extracted using a large scale alkaline protocol as described by Sambrook et al. (1989) and subjected to the polymerase chain reaction (PCR) using two separate oligonucleotide combinations, RK1/RK2 and RK1/RK3 (obtained from M. Cock, École Normale Supérieure de Lyon, France) specifically designed to prime conserved subdomains of the catalytic domain of receptor protein kinases. RK1 (5-ggiggTTTCggiAT^{T}c$_{A}$gTiTT^{A}$_{T}^{T}_{C}$AA^{A}$_{g}$gg - 3'; [SEQ ID NO:18]) served as the forward primer and was constructed based upon a conserved amino acid consensus (GGFGIV^{F}/$_{Y}$KG; [SEQ ID NO:19]) within subdomain I of the catalytic domain. The degeneracy of one reverse primer RK2 (5' - AAiATiC^{T}$_{g}$igCCATiCC^{A}$_{g}$AA^{A}$_{g}^{T}_{C}$ - 3'; [SEQ ID NO:20]) reflects a conserved amino acid consensus (DFGMARIF; [SEQ ID NO:21]) of subdomain VII which closely resembles the SRKs in Brassica. The second reverse oligonucleotide RK3 (5' - A^{g}$_{A}$iA^{g}$_{A}^{T}_{C}$TTigCiA^{A}$_{g}$iCC^{A}$_{g}$AA^{A}$_{g}$TC - 3'; [SEQ ID NO:23]) was generated based upon conserved amino acids (DFGLAKLL; [SEQ ID NO:24]) within subdomain VII prevalent among the RLKs isolated in Arabidopsis. Phagemid DNA was amplified in a reaction mixture containing 1 microliter of excised phagemid DNA, 10x PCR buffer (100mM Tris-HCl pH8.3, 500mM KCl, 15mM $MgCl_2$), 10mM deoxyribonucleotide triphosphate mixture,

10 micromolar of each oligonucleotide primer and 0.5 microliter Tsg polymerase (BioBasics, Canada). The PCR reaction was heated at 95°C for 2 min and amplified for 35 cycles under the following amplification conditions: 1 min at 95°C for denaturation, 1 min 30 sec at 50°C for primer annealing and 1 min at 72°C for synthesis. A final extension cycle of 10 min at 72°C was also incorporated into the amplification program. All PCR products generated of the expected size (420-450 bp) were gel purified, cloned into the pT7Blue plasmid (Novagen, Madison, WI) and introduced into *Escherichia coli* DH5- alpha. Transformants were tested for the presence of an insert and positive clones were sequenced with universal primers (R-20 and U-19) by an ABI automated sequencer (Model 373 STRETCH DNA; Perkin Elmer Corp., Canada Ltd.) using the dideoxychain-terminating method described by Sanger et al. (1977). Sequence analyses performed using DNAsis® software (Hitachi Software, San Bruno, CA) at the nucleotide and amino acid levels.

Please amend the paragraph, commencing at page 54, line 6, as follows.

The 5' end of the PERK1 cDNA was obtained by the procedure for the rapid amplification of cDNA ends originally described by Frohman et al. (1988) using the 5' RACE System, Version 2.0 kit (Gibco-BRL, Gainthersburg, MD). First strand cDNA was synthesized from approximately 300μg of mixed Westar and W1 pistil total RNA using a gene specific primer GSP1 (5'-TAACCAACAAgAgACA-3'; [SEQ ID NO:22]) designed to anneal approximately 300 bp from the 5' end of the PERK1 cDNA (1512 bp) isolated from the library screen. Following cDNA synthesis, the first strand product was purified from unincorporated dNTPs and GSP1 using a GLASS MAX® spin cartridge. A homopolymeric tail was added to the 3' end of the cDNA using TdT (terminal deoxynucleotidyl transferase) and dCTP. Tailed cDNA was amplified using a second gene specific primer GSP2 (5'-CCACTCCCAACTTTCAAC -3'; [SEQ ID NO:25]) designed to anneal 3' to GSP1 with respect to the cDNA, and an abridged anchor primer (Gibco-BRL, Gainthersburg, MD) which annealed to the homopolymeric tail. PCR amplification was carried out for 35 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min and extension at 72°C for

2 min, followed by a final extension cycle for 10 min. A PCR product of the expected size (~1 kb) corresponding to the 5'end of PERK1 was gel purified, cloned into the pT7Blue plasmid (Novagen, Madison, WI) and transformed into *Escherichia coli* DH5- alpha. Confirmation of the 5'RACE product was obtained by plasmid Southern blot analysis as described above and by sequential primer based sequencing.

The paragraph, commencing at page 54, line 25, has been amended as follows.

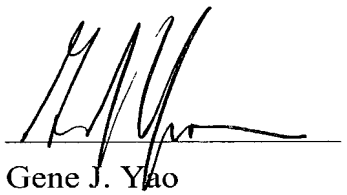
A PCR based approach was used to generate a full length PERK1 cDNA by combining the 5'RACE product cloned into the EcoRV site of pT7Blue with the cDNA isolated from the library screen cloned into the EcoRI/XhoI sites of the pBluescript SK phagemid. A forward primer (5'-ggAAAgCTTgCATgCCTgCAggTCgAC -3'; [SEQ ID NO:26]) containing an internal PstI site was designed to anneal upstream to the EcoRV cloning site of pT7Blue. A reverse primer (5'-CgCCTgCAggTAATACgACTCACTATAggg -3'; [SEQ ID NO:27]) also containing a PstI site was designed based on pBluecsript phagemid sequence immediately 3' to the EcoRI/XhoI cloning site. Full length PERK1 cDNA was generated from a 100 microliter PCR reaction containing 1 microliter (~20ng) of each template (cDNA in pT7Blue and pBluescript phagemid), 10x Pfu Buffer (200mM Tris-HCl pH8.8, 100mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 1% Triton®X-100, 1mg/mlBSA), 10mM dNTPs, 50pmol forward and reverse primers and 1microliter Pfu polymerase (Gibco-BRL, Gainthersburg, MD). The samples were heated to 94°C for 5 min and amplified for 30 cycles with a denaturing cycle of 1 min, a pimer annealing cycle at 53°C for 1 min followed by an extension cycle for 3 min at 72°C. The resulting PCR product of the expected size (~2.2kb) was gel purified and cloned into the PstI restriction site of pBluescript KS (+/-) II. The full length PERK1 cDNA sequence was confirmed by a sequential primer based sequencing approach using both universal and sequence specific primers as previously described. All DNA and protein sequence analysis was performed using the DNAsis® Software (Hitachi Software, San Bruno,CA).

REMARKS

The status of the present application is that applicants have not yet received a first Action on-the-merits. Amendments have been made to the descriptive portion of the specification to bring it in conformity with a Sequence Listing being submitted concurrently. No new matter has been added.

A marked-up version of the changes made to the specification by the current amendment is attached.

Submitted respectfully,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Description

The paragraph, commencing at page 16, line 12, has been amended as follows.

Figure 11. Shows the nucleic acid molecules of [SEQ ID NOS:6 & 7] [[SEQ ID NO.: 6]] and the amino acid sequence of [SEQ ID NO:8] [[SEQ ID NO.: 7]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number AAC98010

A) Genomic Sequence [SEQ ID NO:6]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS:7 & 8]. The transmembrane domain is underlined.

The paragraph, commencing at page 16, line 19, has been amended as follows.

Figure 12. Shows the nucleic acid molecules of [SEQ ID NOS:9 & 10] [[SEQ ID NO.: 8]] and the amino acid sequence of [SEQ ID NO:11] [[SEQ ID NO.: 9]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number AAD15491

A) Genomic Sequence [SEQ ID NO:9]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS:10 & 11]. The transmembrane domain is underlined.

The paragraph, commencing at page 16, line 26, has been amended as follows.

Figure 13. Shows the nucleic acid molecules of [SEQ ID NOS:12 & 13] [[SEQ ID NO.: 10]] and the amino acid sequence of [SEQ ID NO:14] [[SEQ ID NO.: 11]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number CAA18823.

C) Genomic Sequence [SEQ ID NO:12]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

D) Translation of the predicted open reading frame [SEQ ID NOS:13 & 14]. The transmembrane domain is underlined.

The paragraph, commencing at page 17, line 4, has been amended as follows.

Figure 14. Shows the nucleic acid molecules of [SEQ ID NOS:15 & 16] [[SEQ ID NO.: 12]] and the amino acid sequence of [SEQ ID NO:17] [[SEQ ID NO.: 13]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number CAA18590

A) Genomic Sequence [SEQ ID NO:15]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS: 15 & 16]. The transmembrane domain is underlined.

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The isolation of novel *Brassica napus* receptor kinases relied upon the newly [constructedcDNA] constructed cDNA library and involved *in vivo* mass excision of the pBluecsript phagemids from the Uni-ZAP XR vectors as outlined by the manufacturer (Stratagene, La Jolla, CA). Following efficient mass excision, phagemid DNA was extracted using a large scale alkaline protocol as described by Sambrook et al. (1989) and subjected to

the polymerase chain reaction (PCR) using two separate oligonucleotide combinations, RK1/RK2 and RK1/RK3 (obtained from M. Cock, École Normale Supérieure de Lyon, France) specifically designed to prime conserved subdomains of the catalytic domain of receptor protein kinases. RK1 (5- ggiggTTTCggiAT^{T}c$_{A}$gTiTT^{A}$_{T}^{T}_{C}$AA^{A}$_{g}$gg - 3'; [SEQ ID NO:18]) served as the forward primer and was constructed based upon a conserved amino acid consensus (GGFGIV^{F}/$_{Y}$KG; [SEQ ID NO:19]) within subdomain I of the catalytic domain. The degeneracy of one reverse primer RK2 (5' - AAiATiC^{T}$_{g}$igCCATiCC^{A}$_{g}$AA^{A}$_{g}^{T}_{C}$ - 3'; [SEQ ID NO:20]) reflects a conserved amino acid consensus (DFGMARIF; [SEQ ID NO:21]) of subdomain VII which closely resembles the SRKs in Brassica. The second reverse oligonucleotide RK3 (5' - A^{g}$_{A}$iA^{g}$_{A}^{T}_{C}$TTigCiA^{A}$_{g}$iCC^{A}$_{g}$AA^{A}$_{g}$TC - 3'; [SEQ ID NO:23]) was generated based upon conserved amino acids (DFGLAKLL; [SEQ ID NO:24]) within subdomain VII prevalent among the RLKs isolated in Arabidopsis. Phagemid DNA was amplified in a reaction mixture containing 1 microliter of excised phagemid DNA, 10x PCR buffer (100mM Tris-HCl pH8.3, 500mM KCl, 15mM $MgCl_2$), 10mM deoxyribonucleotide triphosphate mixture, 10 micromolar of each oligonucleotide primer and 0.5 microliter Tsg polymerase (BioBasics, Canada). The PCR reaction was heated at 95°C for 2 min and amplified for 35 cycles under the following amplification conditions: 1 min at 95°C for denaturation, 1 min 30 sec at 50°C for primer annealing and 1 min at 72°C for synthesis. A final extension cycle of 10 min at 72°C was also incorporated into the amplification program. All PCR products generated of the expected size (420-450 bp) were gel purified, cloned into the pT7Blue plasmid (Novagen, Madison, WI) and introduced into *Escherichia coli* DH5-alpha. Transformants were tested for the presence of an insert and positive clones were sequenced with universal primers (R-20 and U-19) by an ABI automated sequencer (Model 373 STRETCH DNA; Perkin Elmer Corp., Canada Ltd.) using the dideoxychain-terminating method described by Sanger et al. (1977). Sequence analyses performed using DNAsis® software (Hitachi Software, San Bruno, CA) at the nucleotide and amino acid levels.

The paragraph, commencing at page 54, line 6, has been amended as follows.

The 5' end of the PERK1 cDNA was obtained by the procedure for the rapid amplification of cDNA ends originally described by Frohman et al. (1988) using the 5' RACE System, Version 2.0 kit (Gibco-BRL, Gainthersburg, MD). First strand cDNA was synthesized from approximately 300μg of mixed Westar and W1 pistil total RNA using a gene specific primer GSP1 (5'-TAACCAACAAgAgACA-3'; [SEQ ID NO:22]) designed to anneal approximately 300 bp from the 5' end of the PERK1 cDNA (1512 bp) isolated from the library screen. Following cDNA synthesis, the first strand product was purified from unincorporated dNTPs and GSP1 using a GLASS MAX® spin cartridge. A homopolymeric tail was added to the 3' end of the cDNA using TdT (terminal deoxynucleotidyl transferase) and dCTP. Tailed cDNA was amplified using a second gene specific primer GSP2 (5'-CCACTCCCAACTTTCAAC -3'; [SEQ ID NO:25]) designed to anneal 3' to GSP1 with respect to the cDNA, and an abridged anchor primer (Gibco-BRL, Gainthersburg, MD) which annealed to the homopolymeric tail. PCR amplification was carried out for 35 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min and extension at 72°C for 2 min, followed by a final extension cycle for 10 min. A PCR product of the expected size (~1 kb) corresponding to the 5'end of PERK1 was gel purified, cloned into the pT7Blue plasmid (Novagen, Madison, WI) and transformed into *Escherichia coli* DH5- alpha. Confirmation of the 5'RACE product was obtained by plasmid Southern blot analysis as described above and by sequential primer based sequencing.

The paragraph, commencing at page 54, line 25, has been amended as follows.

A PCR based approach was used to generate a full length PERK1 cDNA by combining the 5'RACE product cloned into the EcoRV site of pT7Blue with the cDNA isolated from the library screen cloned into the EcoRI/XhoI sites of the pBluescript SK phagemid. A forward primer (5'-ggAAAgCTTgCATgCCTgCAggTCgAC -3'; [SEQ ID NO:26]) containing an internal PstI site was designed to anneal upstream to the EcoRV cloning site of pT7Blue. A reverse primer (5'-CgCCTgCAggTAATACgACTCACTATAggg -3'; [SEQ ID NO:27]) also containing a PstI site was designed based on pBluecsript phagemid sequence immediately 3' to the EcoRI/XhoI cloning site. Full length PERK1 cDNA was generated from a 100

microliter PCR reaction containing 1 microliter (~20ng) of each template (cDNA in pT7Blue and pBluescript phagemid), 10x Pfu Buffer (200mM Tris-HCl pH8.8, 100mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 1% Triton®X-100, 1mg/mlBSA), 10mM dNTPs, 50pmol forward and reverse primers and 1microliter Pfu polymerase (Gibco-BRL, Gainthersburg, MD). The samples were heated to 94°C for 5 min and amplified for 30 cycles with a denaturing cycle of 1 min, a pimer annealing cycle at 53°C for 1 min followed by an extension cycle for 3 min at 72°C. The resulting PCR product of the expected size (~2.2kb) was gel purified and cloned into the PstI restriction site of pBluescript KS (+/-) II. The full length PERK1 cDNA sequence was confirmed by a sequential primer based sequencing approach using both universal and sequence specific primers as previously described. All DNA and protein sequence analysis was performed using the DNAsis® Software (Hitachi Software, San Bruno,CA).

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June 24, 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of D. Goring, N. Silva, Y. Haffani
U.S. Application No. 10/086,464
Filed February 28, 2002
Proline-rich Extensin-like Receptor Kinases

Group No. 1645
Examiner NYA

(Atty. Docket No. P 25,762-A USA)

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231, on Monday, June 24, 2002.

Lynn M. White
Lynn M. White

Commissioner for Patents
Washington, D.C. 20231

SECOND PRELIMINARY AMENDMENT UNDER 37 CFR § 1.115

Sir:

Applicants request entry of the following amendments.

In the Description

Please amend the paragraph, commencing after "Cross Reference to Related Application", as follows.

This application is a continuation-in-part of US patent application no. 10/069,304, filed on February 19, 2002, which is a national phase application based on PCT/CA00/00966, filed on August 18, 2000, which claims priority from US patent application no. 60/149,466, filed on August 19, 1999, and US patent application no. 60/159,122, filed on October 13, 1999, and all of the foregoing are incorporated herein by reference in their entirety.

Please amend the paragraph, commencing at page 12, line 9, as follows.

Figure 4A. Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Leaf and Stem Tissue.

Please amend the paragraph, commencing at page 12, line 11, as follows.

(1). Fully expanded leaves were wounded by punching out discs around the perimeter of the leaf blade. Wounds mimic injury inflicted on plants in the field as a result of insect attack or other mechanical damage. Total RNA was extracted at various time intervals after treatment, subjected to Northern blot analysis and probed with full length PERK1 cDNA (bold-face arrow). The blot was re-probed with cyclophilin used as an internal control for even loading (open-face arrow). The graph represents the expression profile of PERK1 in response to wounding corrected against levels of cyclophilin expression. Error bars represent the standard error derived from two independent experiments. Control unwounded leaf tissue

represented by 0 hr time point.

Please amend the paragraph, commencing at page 12, line 19, as follows.

(2). Northern blot showing a time-course induction of PERK1 mRNA accumulation in wounded stem tissue. Total RNA harvested at the indicated time points was blotted and hybridized against the full length PERK1 coding sequence (bold-face arrow). The cyclophilin loading control (open-face arrow) was used to normalize levels of PERK1 mRNA accumulation represented graphically. Error bars represent the standard error derived from two independent experiments. Control unwounded stem tissue represented by 0 hr time point.

Please amend the paragraph, commencing at page 12, line 25, as follows.

Figure 4B. Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Leaf Disc Tissue.

Please amend the paragraph, commencing at page 13, line 6, as follows.

Figure 4C. Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Leaf and Stem Tissue.

Please amend the paragraph, commencing at page 13, line 8, as follows.

(1). Fully expanded leaves were wounded by rubbing the undersides with abrasive sand paper. Total RNA was extracted from the leaf at various time intervals after treatment, subjected to Northern blot analysis and probed with the full length

PERK1 cDNA (bold-face arrow). The blot was reprobed with cyclophilin as an internal control for even loading (open-face arrow). The graph represents the steady state levels of PERK1 mRNA in response to wounding corrected against levels of cyclophilin expression. Control unwounded leaf tissue is represented by the 0hr time point.

Please amend the paragraph, commencing at page 13, line 15, as follows.

(2). Northern blot analysis showing a time-course induction of PERK1 mRNA accumulation in stem wounded by rubbing with abrasive sand paper. Total RNA harvested at the indicated time points was blotted and hybridized against the full length PERK1 cDNA (bold-face arrow). The cyclophilin loading control (open-face arrow) was used to normalize levels of PERK1 mRNA accumulation represented graphically. Control unwounded stem tissue represented by 0hr time point.

Please amend the paragraph, commencing at page 13, line 21, as follows.

Figure 4D. Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Root Tissue. Root tissue from hydroponically grown *B. napus* plants was used to investigate whether levels of PERK1 mRNA increase in response to a wounding stimulus. Roots were wounded by slicing tissue into 3cm sections and incubating on filter paper moistened with 20mM phosphate buffer supplemented with chloramphenicol. Total RNA, extracted at various time intervals after treatment, was subjected to Northern blot analysis and probed with the full length PERK1 cDNA (bold-face triangle). The blot was reprobed with cyclophilin as an internal control for even loading (open-face triangle). The graph represents the expression profile of PERK1 in response to wounding corrected against levels of cyclophilin

expression. Control unwounded root tissue is represented by 0hr time point.

Please amend the paragraph commencing at page 19, line 16, as follows.

In general, plants challenged by mechanical wounded or pathogen attack induce rapid expression of genes (i.e. proteinase inhibitor *(pin)* and pathogenesis related *(PR)* genes respectively) that are expressed locally as well as systemically in unaffected parts of the plant (Yang et al., 1997). Increased levels of extensin transcripts as a result of mechanical wounding have been well established in many other systems (Sauer et al., 1990; Shirsat et al., 1996). For example, in *Brassica napus* leaf and stem tissue, wound induction of PERK1 mRNA accumulation is a very rapid response detectable within 15 min following injury (Figures 4A, 4B, and 4C). Increased levels of PERK1 mRNA were also detected in wounded root tissue within 5 min following treatment (Figure 4D). MeJA (the methyl ester of the plant growth regulator jasmonic acid (JA)) is involved in the signal transduction pathway regulating gene activation upon wounding. Steady state levels of PERK1 mRNA remain unaffected by exogenously applied MeJA (Figure 5) which shows that the inducibility of PERK1 by wounding occurs via a MeJA-independent pathway (Figure 7). Studies conducted by Titarenko et al. (1997) addressing the role of JA in mediating wound responses support the existence of multiple distinct wound signal transduction pathways. Exogenously applied JA was able to induce only a subset of wound responsive genes in Arabidopsis which ultimately resulted in a stronger systemic accumulation in wounded plants. Conversely, a second set of wound responsive genes showing a stronger induction locally in wounded tissue showed no substantial accumulation upon JA treatment. In conjunction with the pattern of PERK1 mRNA accumulation in response to wounding and MeJA, it appears that plants respond to wounding by two distinct wound signal transduction pathways: one

which does not require JA and is primarily responsible for gene activation in the vicinity of the wound site and the other which involves JA perception and activates gene expression both locally and systemically to the wound site (Titarenko et al., 1997).

Please amend the paragraph commencing at page 20, line 10, as follows.

Many of the inducible defense responses are not exclusive to mechanical wounding but are also initiated by pathogen attack. The similarity between responses to wounding and pathogen attack are not surprising since mechanical damage often precedes pathogen infection and conversely, mechanical damage may often result from a pathogen or insect attach (Truernit et al., 1996). Salicylic acid has been implicated in having an important role in the signal transduction pathway leading to systemic acquired resistance (SAR) (Penninckx et al., 1996). Steady state levels of PERK1 mRNA also accumulated in *B. napus* leaf and stem tissue upon exogenous application of 4mM SA (Figure 6). Collectively, the profiles of PERK1 mRNA accumulation in response to wounding, MeJA and SA are not entirely surprising. PERK1 induction is rapid in response to wounding (Figures 4A, 4B, 4C, and 4D) and the lack of PERK1 transcript accumulation in response to MeJA (Figure 5) shows a pathway for wound mediated induction of PERK1 that is independent of MeJA (Figure 7). The pronounced and rapid induction of PERK1 in response to exogenous SA (Figure 6) supports other studies showing that SA is known to inhibit wound response genes that are regulated by a MeJA-dependent pathway (Peña-Cortés et al., 1993; Doares et al., 1995). Therefore, it is unlikely that both MeJA and SA would induce PERK1 mRNA accumulation given that these pathways are known to be antagonistic (Peña-Cortés et al., 1993). Nevertheless, the rapid induction of PERK1 during these treatments shows a role early on in a plant's defense signaling pathway.

Please amend the paragraph commencing at page 50, line 10, as follows.

In order to examine whether PERK1 expression could be influenced by external stimuli, leaf and stem tissue of *B. napus* plants were wounded and the abundance of PERK1 mRNA was determined by standard Northern blot analysis using the full length PERK1 cDNA as a probe (see Methods). Figure 4A shows changes in the steady-state levels of PERK1 mRNA accumulation following injury. PERK1 transcripts in wounded leaf tissue began to accumulate 5 min after wounding, reaching maximal levels within 15 min post injury represented by a 12 fold induction. A 4.5 fold increase in PERK1 mRNA levels was detected 45 min following treatment declining towards basal levels by 2 hr (Figure 4A).

Please amend the paragraph commencing at page 50, line 18, as follows.

A similar profile of PERK1 mRNA steady state levels was obtained for wounded stem tissue (Figure 4A). An accumulation of PERK1 mRNA in stem is evident 5 min following wounding which represents a 3.6 fold induction of this gene. Maximum steady state levels of PERK1 mRNA in stem was achieved 30 min after injury corresponding to a 7 fold induction. PERK1 mRNA levels also accumulate in wounded lead disc tissue as well as following an abrasive wounding treatment (Figures 4B and 4C). Levels of PERK1 mRNA also increase rapidly in the roots of hydroponically grown *Brassica napus* plants (Figure 4D). Therefore, the overall kinetics of PERK1 mRNA accumulation in leaf, stem and root tissue after mechanical wounding is clearly a very rapid response (Figure 4A).

REMARKS

The status of the present application is that applicants have not yet received a first Action on-the-merits. Amendments have been made to the descriptive portion of the specification to bring it in conformity with Substitute Drawings to be submitted in a Reply to Notice to File Missing Parts of Nonprovisional Application. In the Substitute Drawings, Figures 4A, 4B, 4a, 4b(A), 4b(B), and 4c were re-labeled as Figures 4A(1), 4A(2), 4B, 4C(1), 4C(2), and 4D, respectively. In addition, amendments of an editorial nature were made. No new matter has been added.

A marked-up version of the changes made to the specification by the current amendment is attached.

Submitted respectfully,

Gene J. Yao
Reg. No. 47,193
Attorney for Applicants

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Description

The paragraph, commencing after "Cross Reference to Related Application", has been amended as follows.

This application is a continuation-in-part of [a] US patent application no. 10/069,304, [(number not yet assigned)] filed on February 19, 2002, [as Express Mail Label No. EL 930922731US,] which is a national phase application based on PCT/CA00/00966, filed on August 18, 2000, which claims priority from US patent application no. 60/149,466, filed on August 19, 1999, and US patent application no. 60/159,122, filed or October 13, 1999, and all of the foregoing are incorporated herein by reference in their entirety.

The paragraph, commencing at page 12, line 9, has been amended as follows.

[**Figure 4**] **Figure 4A.** Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Leaf and Stem Tissue.

The paragraph, commencing at page 12, line 11, has been amended as follows.

(1).[(A)] Fully expanded leaves were wounded by punching out discs around the perimeter of the leaf blade. Wounds mimic injury inflicted on plants in the field as a result of insect attack or other mechanical damage. Total RNA was extracted at various time intervals after treatment, subjected to Northern blot analysis and probed with full length PERK1 cDNA (bold-face arrow). The blot was reprobed with cyclophilin used as an internal control for even loading (open-face arrow). The graph represents the expression profile of PERK1 in response to wounding corrected against

levels of cyclophilin expression. Error bars represent the standard error derived from two independent experiments. Control unwounded leaf tissue represented by 0 hr time point.

The paragraph, commencing at page 12, line 19, has been amended as follows.

(2). [(B)] Northern blot showing a time-course induction of PERK1 mRNA accumulation in wounded stem tissue. Total RNA harvested at the indicated time points was blotted and hybridized against the full length PERK1 coding sequence (bold-face arrow). The cyclophilin loading control (open-face arrow) was used to normalize levels of PERK1 mRNA accumulation represented graphically. Error bars represent the standard error derived from two independent experiments. Control unwounded stem tissue represented by 0 hr time point.

The paragraph, commencing at page 12, line 25, has been amended as follows.

Figure 4B [4a]. Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Leaf Disc Tissue.

The paragraph, commencing at page 13, line 6, has been amended as follows.

Figure 4C [4b]. Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Leaf and Stem Tissue.

The paragraph, commencing at page 13, line 8, has been amended as follows.

(1). [(A)] Fully expanded leaves were wounded by rubbing the undersides with

abrasive sand paper. Total RNA was extracted from the leaf at various time intervals after treatment, subjected [the] to Northern blot analysis and probed with the full length PERK1 cDNA (bold-face arrow). The blot was reprobed with cyclophilin as an internal control for even loading (open-face arrow). The graph represents the steady state levels of PERK1 mRNA in response to wounding corrected against levels of cyclophilin expression. Control unwounded leaf tissue is represented by the 0hr time point.

The paragraph, commencing at page 13, line 15, has been amended as follows.

(2). [**(B)**] Northern blot analysis showing a time-course induction of PERK1 mRNA accumulation in stem wounded by rubbing with abrasive sand paper. Total RNA harvested at the indicated time points was blotted and hybridized against the full length PERK1 cDNA (bold-face arrow). The cyclophilin loading control (open-face arrow) was used to normalize levels of PERK1 mRNA [mRN] accumulation represented graphically. Control unwounded stem tissue represented by 0hr time point.

The paragraph, commencing at page 13, line 21, has been amended as follows.

Figure 4D [**4c**]. Wound-Inducible Accumulation of PERK1 mRNA in *Brassica napus* Root Tissue. Root tissue from hydroponically grown *B. napus* plants was used to investigate whether levels of PERK1 mRNA increase in response to a wounding stimulus. Roots were wounded by slicing tissue into 3cm sections and incubating on filter paper moistened with 20mM phosphate buffer supplemented with chloramphenicol. Total RNA, extracted at various time intervals after treatment, was subjected to Northern blot analysis and probed with the full length PERK1 cDNA

(bold-face triangle). The blot was reprobed with cyclophilin as an internal control for even loading (open-face triangle). The graph represents the expression profile of PERK1 in response to wounding corrected against levels of cyclophilin expression. Control unwounded root tissue is represented by 0hr time point.

The paragraph commencing at page 19, line 16, has been amended as follows.

In general, plants challenged by mechanical wounded or pathogen attack induce rapid expression of genes (i.e. proteinase inhibitor *(pin)* and pathogenesis related *(PR)* genes respectively) that are expressed locally as well as systemically in unaffected parts of the plant (Yang et al., 1997). Increased levels of extensin transcripts as a result of mechanical wounding have been well established in many other systems (Sauer et al., 1990; Shirsat et al., 1996). For example, in *Brassica napus* leaf and stem tissue, wound induction of PERK1 mRNA accumulation is a very rapid response detectable within 15 min following injury [(Figure 4, 4a-b)] (Figures 4A, 4B, and 4C). Increased levels of PERK1 mRNA were also detected in wounded root tissue within 5 min following treatment [(Figure 4c)] (Figure 4D). MeJA (the methyl ester of the plant growth regulator jasmonic acid (JA)) is involved in the signal transduction pathway regulating gene activation upon wounding. Steady state levels of PERK1 mRNA remain unaffected by exogenously applied MeJA (Figure 5) which shows that the inducibility of PERK1 by wounding occurs via a MeJA-independent pathway (Figure 7). Studies conducted by Titarenko et al. (1997) addressing the role of JA in mediating wound responses support the existence of multiple distinct wound signal transduction pathways. Exogenously applied JA was able to induce only a subset of wound responsive genes in Arabidopsis which ultimately resulted in a stronger systemic accumulation in wounded plants. Conversely, a second set of wound responsive genes showing a stronger induction

locally in wounded tissue showed no substantial accumulation upon JA treatment. In conjunction with the pattern of PERK1 mRNA accumulation in response to wounding and MeJA, it appears that plants respond to wounding by two distinct wound signal transduction pathways: one which does not require JA and is primarily responsible for gene activation in the vicinity of the wound site and the other which involves JA perception and activates gene expression both locally and systemically to the wound site (Titarenko et al., 1997).

The paragraph commencing at page 20, line 10, has been amended as follows.

Many of the inducible defense responses are not exclusive to mechanical wounding but are also initiated by pathogen attack. The similarity between responses to wounding and pathogen attack are not surprising since mechanical damage often precedes pathogen infection and conversely, mechanical damage may often result from a pathogen or insect attach (Truernit et al., 1996). Salicylic acid has been implicated in having an important role in the signal transduction pathway leading to systemic acquired resistance (SAR) (Penninckx et al., 1996). Steady state levels of PERK1 mRNA also accumulated in *B. napus* leaf and stem tissue upon exogenous application of 4mM SA (Figure 6). Collectively, the profiles of PERK1 mRNA accumulation in response to wounding, MeJA and SA are not entirely surprising. PERK1 induction is rapid in response to wounding [(Figure 4, 4a-c)] (Figures 4A, 4B, 4C, and 4D) and the lack of PERK1 transcript accumulation in response to MeJA (Figure 5) shows a pathway for wound mediated induction of PERK1 that is independent of MeJA (Figure 7). The pronounced and rapid induction of PERK1 in response to exogenous SA (Figure 6) supports other studies showing that SA is known to inhibit wound response genes that are regulated by a MeJA-dependent pathway (Peña-Cortés et al., 1993; Doares et al., 1995). Therefore, it is unlikely that

both MeJA and SA would induce PERK1 mRNA accumulation given that these pathways are known to be antagonistic (Peña-Cortés et al., 1993). Nevertheless, the rapid induction of PERK1 during these treatments shows a role early on in a plant's defense signaling pathway.

The paragraph commencing at page 50, line 10, has been amended as follows.

In order to examine whether PERK1 expression could be influenced by external stimuli, leaf and stem tissue of *B. napus* plants were wounded and the abundance of PERK1 mRNA was determined by standard Northern blot analysis using the full length PERK1 cDNA as a probe (see Methods). Figure [4] 4A shows changes in the steady-state levels of PERK1 mRNA accumulation following injury. PERK1 transcripts in wounded leaf tissue began to accumulate 5 min after wounding, reaching maximal levels within 15 min post injury represented by [an 12fold] a 12 fold induction. A 4.5 fold increase in PERK1 mRNA levels was detected 45 min following treatment declining towards basal levels by 2 hr [(Figure 4)] (Figure 4A).

The paragraph commencing at page 50, line 18, has been amended as follows.

A similar profile of PERK1 mRNA steady state levels was obtained for wounded stem tissue [(Figure 4)] (Figure 4A). An accumulation of PERK1 mRNA in stem is evident 5 min following wounding which represents a 3.6 fold induction of this gene. Maximum steady state levels of PERK1 mRNA in stem was achieved 30 min after injury corresponding to a 7 fold induction. PERK1 mRNA levels also accumulate in wounded lead disc tissue as well as following an abrasive wounding treatment [(Figure 4a, 4b)] (Figures 4B and 4C). Levels of PERK1 mRNA also increase rapidly in the roots of hydroponically grown *Brassica napus* plants [(Figure

4c)] (Figure 4D). Therefore, the overall kinetics of PERK1 mRNA accumulation in leaf, stem and root tissue after mechanical wounding is clearly a very rapid response [(Figure 4)] (Figure 4A).